

AMENDMENTS

In the Specification

Please replace the paragraph beginning on page 6, line 14, with the following rewritten paragraph:

Figure 12: Sequence similarity analysis using 'sim' and llanview mathematical and software tools (Duret, Comput. Appl. Biosci." 12 (1996), 507-510). In each computation the gap open penalty was set to 12, and gap extension penalty 4. Comparison matrix for A was 'PAM40', and BLOSUM62 for B and C respectively (see Duret, Comput. Appl. Biosci. 12 (1996), 507-510; Huang, Comput. Appl. Biosci. 8 (1992), 155-165; Huang, Comput. Appl. Biosci. (1990) 6, 373-381). The similarity score threshold was 70% in A, and 40% in B and C respectively. The highlighted blocks shown on each protein scheme represent sequence homologies of >80% in A, and > 62% in B and C. Note that in MEPE versus DSSP (A), there are five homology blocks in DSSP of >80% sequence similarity to a single motif in MEPE (DSSESSDSGSSSES) (SEQ ID NO.: 26). A similar sequence homology is also apparent for DMA-1 and OPN versus MEPE (B and C) and the MEPE is a feature of all three proteins.

Please replace the paragraph beginning on page 9, line 1, with the following rewritten paragraph:

The present invention also describes the characterization and cloning of a gene that is a candidate for the above-described tumour-derived phosphaturic factor and that is named phosphatonin or MERE (Metastatic-tumour Excreted Phosphaturic-Element). To summarize, expression screening of a A, ZAPII-cDNA library constructed from mRNA extracted from an OH0 tumour using antisera specific to tumor conditioned media (TCM) phosphaturic-factor was used. The protein is glycosylated and resolves as two bands on SDS-PAGE electrophoresis (58-60 kDa), with evidence of possible splicing or post translational cleavage. The cloned cDNA codes for a protein of 430 residues (SEQ ID NO: 2) and 1655 bp in length (SEQ ID NO: 1). The entire 3' end of the gene is present, with part of the 5' end missing. The fusion protein containing 10 residues of f3-galactosidase is highly potent at inhibiting Na⁺ dependent phosphate co-transport in a human renal cell line (CL8). Secondary structure prediction confirms that the protein is highly hydrophilic with small localized regions of hydrophobicity and no cysteine residues. A number of helical regions are present, with two distinct N-glycosylation motifs at the carboxy-terminus. A key feature is the presence of a cell attachment sequence in the same structural context found in osteoponlin. Proteolytic-sites adjacent to this motif may result in altered receptor specificity for specific integrins as found in osteopontin. Screening of the trembl database with MEPE sequence also demonstrated sequence homology with Dentin phosphoryn (DPP). In particular there is

striking localized residue homology at the C'-terminus of MEPE with DPP, dentin-matrix protein-1 (DMA-1) and osteopontin (OPN). This region of MEPE contains a recurring series of aspartate and serine residues (DDSESSSDGSSSESD) (SEQ ID NO.: 27), with 80%, 65 % and 62% homology with DSP, DMA-1 and OPN respectively. Moreover, when residue physicochemical character is considered this homology rises to 93%, suggesting a shared or related biological-functionality. It is also of note that this structural motif overlaps a casein kinase II phosphorylation motif in MEPE. Skeletal casein kinase II activity is defective in rickets, and results in under phosphorylation of osteopontin (Rifas, *Calcif. Tissue Int.* 61 (1997), 256-259). The casein kinase II defect has thus been proposed to play a role in the under-mineralization of bone matrix (Rifas, loc. cit).

Please replace the paragraph beginning on page 37, line 17, with the following rewritten paragraph:

Using the computer program GCG-Peptide-structure (Rice, Programme Manual for the EGCG package, Cambridge, CB10 1RQ England: Hinxton Hall; 1995) available from the Human Genome Resource Centre (<http://www.hgmp.mrc.ac.uk/homepage.htm>). SEQ ID NO:2 was found antigenic at amino acids: regions shown in Figure 4. Thus, these regions could be used as epitopes to produce antibodies against the protein encoded by SEQ ID No: 1.

Please replace the paragraph beginning on page 45, line 14 and ending on page 47, line 20, with the following rewritten paragraph:

There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Phosphatonin related polynucleotides -C (genomic and/or cDNA) can be used to carry out restriction analysis as described in detail (Rowe, *Hum. Genet.* 94:5 (1994), 457-467; Benham, *Genomics* 12 (1992), 368-376; Gillett, *Ann. Hum. Genet.* 60(3) (1996), 201-211; Rowe, *Nucleic Acids Res.* 22(23) (1994), 5135-5136). In particular, the use of microsatellites (Rowe, *Hum. Genet.* 94:5 (1994), 457-467; Rowe, *Nucleic Acids Res.* 22(23) (1994), 5135-5136; Rowe, *Hum. Genet.* 93 (1994), 291-294; Rowe, *Hum. Genet.* 91 (1993), 571-575; Rowe, *Hum. Genet.* 97 (1996), 345-352; Rowe, *Hum. Genet.* 89 (1992), 539-542), and the isolation of informative markers using irradiation- fusion-gene-transfer hybrids and ALU-PCR (Benham, *Genomics* 12 (1992), 368- 376) will enable the rapid isolation of highly informative methods for the screening of phosphatonin and derivative inherited diseases. The above methodologies have been particularly successful in the mapping and localization of the PHEX gene (MERE is proposed to a PHEX substrate), and extensive mutation analysis has revealed structural regions and

motifs prerequisite for PHEX bio-activity (Rowe, Hum. Mol. Genet. 6 (1997), 539-549; Rowe, Exp. Nephrol. 5 (1997), 355-363; Rowe, Current Opinion in Nephrology & Hypertension 7(4) (1998), 367-376; Rowe, Clinical and Experimental Nephrology 2(3) (1998), 183-193), these same approaches can be used for phosphatonin. More recently powerful genome-wide linkage and screening techniques have been developed that rely on single nucleotide polymorphisms (SNP's), and the use of a combination of gel-based sequencing and high-density variation-detection DNA chips (Wang, Science 280 (1998), 1077-1082). Recently SNP data has been made available on the internet via the Center for Genome Research at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, USA (Whitehead-MIT) at

<http://www.genome.wi.mit.edu/SNP/human/index.html>. This powerful new oligonucleotide- array based methodology will be the future route for molecular expression analysis, polymorphism and genotyping, and disease management (Wang, Science 280 (1998), 1077-1082; Chee, Science 274 (1996), 610-614; Gentalen, Nucleic Acids Res. 27 (1999), 1485-1491; Hacia, Nucleic Acids Res. 26 (1998), 3865-3866; Lipshutz, Nat. Genet. 21 (1999), 20-24; Fan, Eur. J. Hum. Genet. 6 (1998), 134). Given the sequence information for MEPE in this application the above new approaches and technology will be used to address the areas described. The sequence may be mapped to a particular chromosome or to a M:; specific region of the chromosome using well known techniques. These include *in situ* hybridization to chromosomal spreads, flow-sorted chromosomal preparations, or artificial chromosome constructions such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price (Blood Rev. 7 (1993), 127-134) and Trask (Trends Genet. 7 (1991), 149-154). The technique of fluorescent *in situ* hybridization of chromosome spreads has been described, among other places, in Verma, (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY. Fluorescent *in situ* hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Extensive mapping data accessible to the scientific community can be found on the internet at sites sponsored by the Human-Genome-Mapping-Project United Kingdom (HGMP-RC)

<http://www.hgmp.mrc.ac.uk/homepage.html>, the National Collection of biological information (NCBI) sponsored by the National Institute of Health USA (NIH), <http://www.ncbi.nlm.nih.gov/>, also the Center for Genome Research at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, USA (Whitehead-MIT)

<http://www.genome.wi.mit.edu/>. Moreover, extensive microsatellite-maps and related mapping tools covering the entire human genome can also be accessed via Genethon (French Government sponsored database)

http://www.genethon.fr/genethon_en.html. Seminal maps have also been published in Science and Nature (see, for example, Dib, Nature 380 (1996), 152-154), but for up to date data the internet sites should be consulted.

Correlation between the location of the gene encoding a phosphatonin polypeptide of the invention on a physical chromosomal map and a specific feature, e.g. a hypo- or hyperphosphatemic disease may help to delimit the region of DNA associated with this feature. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals. Furthermore, the means and methods described herein can be used for marker-assisted animal breeding. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier or affected individuals.

Please replace the Table 1 on page 76, with the following rewritten Table 1:

TABLE 1

	Site (on Figure 8)	Motif
Protein Kinase C phosphorylation	8-10	SNK
	77-79	TPR
	118-120	THR
	203-205	TKK
	228-230	TAK
	311-313	STR
	312-314	TRK
	319-321	SNR
	384-386	STR
	403-405	SNR
Casein Kinase II phosphorylation	408-410	SSR
	409-411	SRR
	8-11	SNKE (SEQ ID NO.: 28)
	139-142	SDFE (SEQ ID NO.: 29)
	177-180	TGPD (SEQ ID NO.: 30)
	194-197	SEAE (SEQ ID NO.: 31)
	199-202	THLD (SEQ ID NO.: 32)
	224-227	TRDE (SEQ ID NO.: 33)
	228-231	TAKE (SEQ ID NO.: 34)
	238-241	SLVE (SEQ ID NO.: 35)
	325-328	TLNE (SEQ ID NO.: 36)

		<u>36)</u>
	423-426	SSSE (SEQ ID NO.: <u>37)</u>
	425-428	SESD (SEQ ID NO.: <u>38)</u>
	427-430	SDGD (SEQ ID NO.: <u>39)</u>
CAMP- & cGMP-dependent protein kinase phosphorylation	405-408	RRFS (SEQ ID NO.: <u>40)</u>
Tyrosine Kinase phosphorylation	40-47	KLHDQEEY (SEQ ID NO.: 41)
Myristoylation	16-21	GLRMSI (SEQ ID NO.: 42)
	143-148	GSGYTD (SEQ ID NO.: 43)
	119-224	GNTIGT (SEQ ID NO.: 44)
	266-271	GSQNAH (SEQ ID NO.: 45)
	291-296	GSSDAA (SEQ ID NO.: 46)
	315-320	GVDHSN (SEQ ID NO.: 47)
	389-394	GMPQGKHGRK (SEQ ID NO.: 48)
Amidation	370-373	HGRK (SEQ ID NO.: 49)
RGD	152-154	RGD
Gycosaminoglycan Attach. Site	161-165	SGDG (SEQ ID NO.: 50)
Asu-Glycosylation	382-386	NNST (SEQ ID NO.: 51)
	383-387	NSTR (SEQ ID NO.: 52)

Please insert the attached "Sequence Listing" as separately numbered pages 1 - 12 after the abstract.